

2006 Non-tradewind Season

EFFLUENT BIOASSAY TEST RESULTS

March 2006 Sampling



TECHNICAL MEMORANDUM



BIOASSAY TESTING - JOINT CANNERY OUTFALL EFFLUENT MARCH 2006 SAMPLING

Prepared For:

StarKist Samoa (NPDES Permit AS0000019)

COS Samoa Packing (NPDES Permit AS0000027)

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Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in March 2006. The testing is required by the NPDES Permits that became effective in January 2001. This is the eleventh required semi-annual test required by the current permits and the twenty-seventh test conducted since toxicity testing of the Joint Cannery Outfall effluent began in 1993¹.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the combined joint cannery effluent discharge into Pago Pago Harbor.

¹ Testing was not conducted during 1999. Extra tests using two organisms were conducted in March 1995 and February 1996.

Study Approach

The U.S. Environmental Protection Agency (USEPA) has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from (USEPA have been incorporated into the sampling and sample handling standard operating procedures (SOP) or have been incorporated into the procedures used by the laboratory doing the test. The comments, responses, and SOP have been documented in previous reports.

The NPDES permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, the permit specifies the substitute species, *Mysidopsis bahia*, which now has been renamed *Americamysis bahia*. For the March 2006 sampling, *Penaeus vannami* was not available and *Americamysis bahia* was used.

Effluent samples were collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The acute effluent bioassay test was conducted using a combined, flow-weighted, composite effluent sample made up from the effluent samples from both canneries, as allowed by the NPDES permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 09:00 on 28 February 2006 and 06:00 on 1 March 2006, a 24-hour flow-weighted composite sample of final effluent was collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into 1-gallon plastic cubitainers at each cannery. Samples were collected at approximately three-hour intervals over the 24-hour period. The samples were stored on ice or in a refrigerator until the completion of the 24-hour sampling period. After all samples were collected a 5-gallon flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL to the testing laboratory. The chain-of-custody form and the DHL waybill are provided in Attachment I.

Table 1 StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing March 2006														
COS Samoa Packing StarKist Samoa COS Samoa Packing Samoa Packing Percent														
Number	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)	Percent of Total Flow	Of Total Flow								
28 February 2006														
1	09:00	0.88	09:00	1.98	3.57	8.02								
2	12:00	0.84	12:00	2.63	3.40	10.66								
3	15:00	0.84	15:00	2.61	3.40	10.58								
4	18:00	0.80	18:00	1.94	3.24	7.86								
5	21:00	0.80	21:00	1.95	3.24	7.91								
			1 March 2006											
6	00:00	0.80	00:00	2.16	3.24	8.77								
7	03:00	0.88	03:00	2.53	3.57	10.25								
8	06:00	0.88	06:00	2.15	3.57	8.71								
Total		6.72 ^A		17.95 ^A	27.2%	72.8%								
Mean		0.84		2.24		0.12 11.93 21.2% 12.0%								

Bioassay Testing Procedures

the calculated mean.

EnviroSystems, Inc. located in Hampton, New Hampshire conducted the bioassay tests. The testing procedures and results of the bioassay tests are provided in the laboratory report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to the (USEPA) document Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA-821-R-02-012), 2002 as the source of methods for conducting the test. The

bioassay test was conducted considering and including USEPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and the test temperature was to be held at a nominal 20 °C, with actual temperatures ranging between 19°C and 21°C. Salinity was adjusted to 25 ppt at the start of the test and ranged during the test between 24 and 28 ppt.

Demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD) and a delayed dissolved oxygen demand spike (DDOD) had been discussed and documented in previous technical memoranda, which describe the first two tests conducted in 1993. Therefore, all of the bioassay test chambers should have been continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO)². The test should also be renewed with pre-oxygenated effluent sample at 48 hours. However, the laboratory failed to follow these procedures during this test and the DO went to lethally low levels in the higher effluent concentrations within the twenty four hours of the test. (See the Laboratory Report in Attachment II.)

Because of the low DO levels the toxicity of the effluent could not be determined. The apparent toxicity caused by the low DO levels is higher than the actual toxicity. Since the results of the test show that the apparent toxicity (although masked by low DO levels) is still reduced to acceptable levels well within the ZID, the results of the test are being reported below. However, the canneries intend to re-run the test. The new results will be reported when available.

Bioassay tests were carried out for effluent concentrations of 100, 75, 50, 25, 12.5, and 6.25 percent as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. Water quality data are provided in the Laboratory Report (Attachment II). Reference toxicant tests using sodium dodecyl sulfonate (SDS) are conducted regularly by ESI with the last one completed on 4 January 2006 and results were within the acceptable range based on the 20 most recent laboratory tests.

² The high initial dilution of the actual effluent discharge (>100:1) into the Harbor, in a very short time, eliminates any concern about IDOD effects in the receiving water.

Summary Results: Americamysis bahia Effluent Bioassay

All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC₅₀ for the effluent tested was 35.6% percent. The no observable effects concentration (NOEC) for the 96-hour bioassay was 25 percent and the least observable effects concentration (LOEC) was 50 percent. Results on a daily basis are summarized in Table 2.

Table 2 StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results March 2006 Sampling								
Evnonura Timo		Parameter						
Exposure Time	LC 50	NOEC	LOEC					
24 hours	>100%	100%	>100%					
48 hours	>63.3%	50%	75%					
72 hours >35.8% 25% 50%								
96 hours	>35.6%	25%	>50%					

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the March 2006 sampling compared to the previous bioassay tests. The LC₅₀, NOEC, and LOEC are within the range obtained from previous tests where Americamysis bahia (Mysidopsis bahia) was used in place of Penaeus vannami. Figure 1 summarizes the LC₅₀ for the mysid and penaeid tests done since February 1993. Figure 2 presents the range of LC₅₀ results for mysids tests conducted since 1994. There is some variability observed in test results. The March 2006 test results are among the highest LC₅₀ values recorded for this organism, but because of the DO problems during the test are lower than the results from the last 2½ years. Higher LC₅₀ values indicate lower whole effluent toxicity. There is a possible trend toward lower toxicity (higher LC₅₀) with time (see Figure 2).

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for March 2006 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an LC₅₀ of 35.6 percent after 96 hours of exposure, which was observed in March 2006, corresponds to a dilution of 2.8:1, which is achieved within one second and within one meter of the discharge point. The discharge is located in about 180 feet of water

and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

An LC₅₀ of 35.6 percent corresponds to 2.8 acute toxicity units (TU_a). A dilution of less than 10:1 will reduce the toxicity to less than 0.3 TU_a which is considered the acceptable level for the protection of aquatic life. The JCO achieves an initial dilution, under critical conditions of greater than 300:1. Therefore, at the edge of the zone of initial dilution (ZID) the acute toxicity is 0.11 TU_a for the LC₅₀ documented in this test. Since the test appears to be compromised by low DO depressions during the test the actual toxicity at the edge of the ZID is expected be even lower.

Table 3 StarKist Samoa and COS Samoa Packing **Combined Effluent Bioassay Results**

- .		Parameters						
Date	Species	LC 50	NOEC	LOEC				
2/93	Penaeus vannami	4.8%1/	3.1%	6.25%				
10/93	Penaeus vannami	15.67%	3.1%	6.25%				
2/94	Penaeus vannami	15.76%	<1.6%	1.6%				
10/94	Mysidopsis bahia ²	31.2%	25%	50%				
3/95	Penaeus vannami	14.8%	6.25%	12.5%				
3/95	Mysidopsis bahia ³	10.8%	6.25%	12.5%				
2/96	Penaeus vannami	>50%	>50%	>50%				
2/96	Mysidopsis bahia ³	28.36%	12.5%	25%				
3/96	Penaeus vannami	44.4%	25%	50%				
11/96	Penaeus vannami	7.11% /	3.1%	6.25%				
03/97	Penaeus vannami	39.36%	12.5%	25%				
09/97	Penaeus vannami⁴	12.3%	6.25%	12.5%				
06/98	Mysidopsis bahia ²	17.2%	6.25%	12.5%				
11/98	Mysidopsis bahia ²	15%	6.25%	12.5%				
02/00	Mysidopsis bahia ²	20%	6.25%	12.5%				
08/00	Mysidopsis bahia ²	17.1%	3.1%	6.25%				
03/01	Americamysis bahia ^{2,5}	13.8%	12.5%	25%				
10/01	Americamysis bahia ^{2,6}	37.5%	25%	50%				
3/02	Americamysis bahia ^{2,6}	16.1%	12.5%	25%				
8/02	Americamysis bahia ^{2,6}	10.23%	6.25%	12.5%				
03/03	Americamysis bahia ^{2,6}	28.4%	25%	50%				
08/03	Americamysis bahia ^{2,6}	43.2%	25%	50%				
02/04	Americamysis bahia ^{2,6}	>50%	50%	>50%				
09/04	Americamysis bahia ^{2,6}	>50%	50%	>50%				
03/05	Americamysis bahia ^{2,6}	48.5%	25%	50%				
08/05	Americamysis bahia ^{2,8}	>50%	50%	>50%				
03/06	Americamysis bahia ^{2,6}	35.6% ⁷	25%	50%				

^{&#}x27;The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

²Mysidopsis bahia used as substitutes because Penaeus vannami not available: as directed and approved

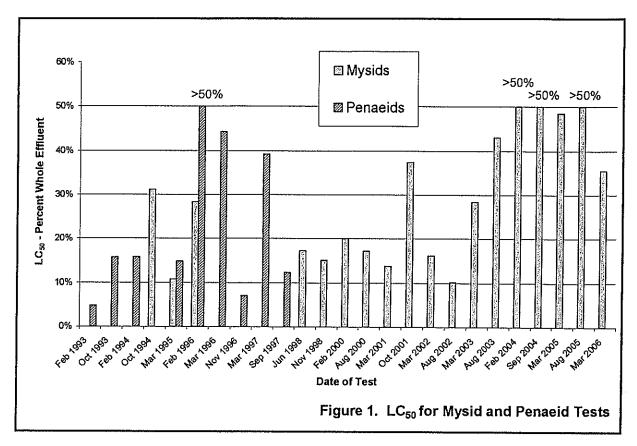
³Mysidopsis bahia used in addition to Penaeus vannami as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.

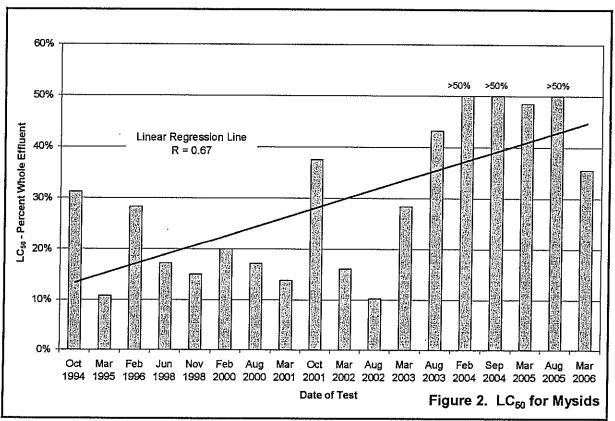
⁴Stage 1 (3 mm) *Penaeus vannami* were used for testing because older Stage 7 and 8

⁽⁸⁻¹⁰ mm) *Penaeus vannami* were not available.

Mysidopsis bahia renamed *Americamysis bahia*. Results indicate increased toxicity because of low DO in renewal concentrations as renewal water was not aerated prior to use ⁸ Mysidopsis bahia renamed Americamysis bahia

⁷Results for this test depressed because aeration was not provided (see text).





ATTACHMENT I

Chain-of-Custody

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ATTACHMENT II

EnviroSystems, Inc. Laboratory Report

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT MARCH 2006

American Samoa Joint Cannery Outfall

Prepared For

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March 2006 Reference Number CH2M-Samoa14326-06-03

STUDY NUMBER 14326

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from March 8-12, 2006 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assay was conducted using the marine species, *Americamysis bahia*.

Acute Toxicity Evaluation

Species	Exposure	LC-50	NOEC	LOEC
Americamysis bahia	24-Hours	>100%	100%	>100%
	48-Hours	63.3%	50%	75%
	72-Hours	35.8%	25%	50%
	96-Hours	35.6%	25%	50%

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT MARCH 2006

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test organisms are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test organisms. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of an acute toxicity test conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (2002) and involved conducting 96 hour acute static renewal toxicity tests with the marine species, *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire in accordance with the provisions of the NELAC Standards (2000).

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

Every attempt was made to acquire the species, *Penaeus vannami*, as this is the preferred organism under the Cannery's permit. ESI was unable to obtain reasonably priced *P. vannami*. Due to the exorbitant expense, the decision was made to use an alternate species, *Americamysis bahia*.

A. bahia, ≤5 days old, were from cultures maintained at Aquatic Research Organisms. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

American Samoa Joint Cannery Effluent Biomonitoring Program, March 2006.

Study Number 14326. Page 3 of 10

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "06NT." Sample collection information is provided in Table 1. Upon receipt, the sample was stored at 4°C. All sample material used in the assay was warmed to 20±1°C prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L, TRC dechlorination with sodium thiosulfate was not required (EPA 2002). Aliquots of the undiluted effluent sample were collected for ammonia analysis when the sample arrived and again prior to renewal. Upon arrival, the effluent sample had a salinity of 11‰. Salinity of the effluent was increased to 25‰ by the addition of artificial sea salts. Test concentrations for the assays were 100%, 75%, 50%, 25%, 12.5%, and 6.25% effluent with a laboratory water diluent control.

The dilution water used in this assay was collected from the sea water system at ESI. The water is pumped in daily from the Hampton Estuary on the flood tide, filtered through a high volume sand filter, and stored in 3000 gallon polyethylene tanks. The water is classified as Class SA-1 by the State of New Hampshire, and has been used to culture test organisms for over 20 years. Sea water used in the assay had a salinity of 25±2‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity test was conducted at 20±2°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates, with 10 organisms/replicate. Survival, dissolved oxygen, pH, salinity and temperature were measured daily in all replicates. Test solutions were renewed after 48 hours using effluent from the start sample. Mysid shrimp were fed daily with <24 hour old brine shrimp.

2.5 Data Analysis

At 24 hour intervals, survival data was analyzed to assess toxicity using CETIS, Comprehensive Environmental Toxicity Testing System, software. The program computes acute exposure endpoints based on EPA decision tree guidelines specified in individual test methods. For acute exposure endpoints statistical significance was accepted at \approx <0.05.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. See Table 2 for details.

3.0 RESULTS

Results of the acute exposure bioassay conducted using the mysid shrimp, *A. bahia*, are summarized in Table 3. Effluent and dilution water characteristics are presented in Table 4. Table 5 provides a summary of historic data associated with the discharge. Support data are included in Appendix A.

3.1 Acute Toxicity Test - Americamysis bahia

Minimum test acceptability criteria require ≥90% survival in the control concentration. As the laboratory water diluent control met or exceeded this protocol specification, results associated with the assay indicate healthy test organisms and that the dilution water had no adverse impact on the outcome of the assay. These data are considered as valid for evaluating impacts associated with the effluent sample.

Table 3 provides a summary of the acute exposure data and results.

3.2 Summary

The salinity adjusted effluent sample collected from the American Samoa Joint Cannery Outfall did exhibit signs of acute toxicity to the mysid shrimp, *Americamysis bahia*, during the 96 hour exposure period.

4.0 LITERATURE CITED

- APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Washington D.C.
- National Environmental Laboratory Accreditation Conference: Quality Systems. Chapter 5. June 2000.
- Stephan, C. 1982. Documentation for Computing LC-50 Values with a Mini Computer. Unpublished.
- US EPA. 2002. Attachment G: NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting Tips and Common Pitfalls. Dated December 2002. US EPA Region I Offices, Boston, Massachusetts.
- U.S. EPA. 2002. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fifth Edition. EPA-821-R-02-012.

TABLE 1. Summary of Sample Collection Information.

American Samoa Joint Cannery Outfall Effluent Evaluation. March 2006.

Sample		Collection)	Recei	pt	Arrival
Description	Туре	Date	Time	Date	Time	Temp °C
EFFLUENT	Comp	02/28-03/01/06	ND	03/08/06	1240	0

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation, March 2006.

Date	E	ndpoint	Value	Historic Mean/ Central Tendency	Acceptable Range	Reference Toxicant
A. bahia						
01/04/06	Survival	LC-50	17.6	20.1	14.8 - 25.3	SDS (mg/L)

Means and Acceptable Ranges based on the most recent 20 reference toxicant assays

TABLE 3A. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2006.

Concentration	Exposure		Re	plicate	s		Mean	Standard	Coefficient
% Effluent	Exposure	A	В	С	D	E	Wicari	Deviation	of Variation
Lab Control	Start	10	10	10 .	10	10	100%	0.000	0.00%
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	9	10	9	10	9	94%	0.055	5.85%
	96-Hours	8	10	9	10	9	92%	0.084	9.13%
6.25%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	9	10	10	10	10	98%	0.045	4.59%
	72 Hours	9	10	10	10	10	98%	0.045	4.59%
	96-Hours	9	10	8	10	10	94%	0.110	11.70%
12.5%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	9	10	8	10	94%	0.089	9.47%
25%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	9	10	98%	0.045	4.59%
	96-Hours	9	10	10	7	10	92%	0.130	14.13%
50%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	0	9	78%	0.438	56.15%
	72 Hours	1	0	0	0	0	2%	0.045	225.00%
	96-Hours	1	0	0	0	0	2%	0.045	225.00%
75%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	3	5	0	7	7	44%	0.297	67.50%
	72 Hours	0	0	0	0	0	0%	0.000	0.00%
	96-Hours	0	0	0	0	0	0%	0.000	0.00%
100%	24-Hours	10	9	10	8	9	92%	0.084	9.13%
	48-Hours	0	0	0	0	0	0%	0.000	0.00%
	72 Hours	0	0	0	0	0	0%	0.000	0.00%
	96-Hours	0	0	0	0	0	0%	0.000	0.00%

TABLE 3B. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2006.

SUMMARY	OF ENDPOINTS	
50	METHOD	

Exposure Period	LC-50 (95% Limits)	METHOD	NOEC	LOEC
24 Hours	>100%	Direct Observation	100%	>100%
48 Hours	63.3% (58.3-68.7)	Trimmed Spearman-Karber	50%	75%
72 Hours	35.8% (35.0-36.6)	Trimmed Spearman-Karber Direct Observation	25%	50%
96 Hours	35.6% (34.6-36.6)	Trimmed Spearman-Karber Direct Observation	25%	50%

TABLE 4. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2006.

PARAMETER	UNITS	100% EFFLUENT	50% EFFLUENT	DILUENT
Salinity - As Received	%	11	-	25
Salinity - After Salinity Adjustment	%	25	25	-
pH - As Received	SU	6.46	-	7.87
pH - After Salinity Adjustment	SU	7.07	7.22	-
TRC - As Received	mg/L	<0.05	-	<0.05
Dissolved Oxygen - As Received	mg/L	1.3	_	-
Dissolved Oxygen - After Aeration	mg/L	3.6	5.8	7.3
Ammonia - As Received	mg/L as N	23.4	-	<0.1
Unionized Ammonia - As Received	mg/L as N	0.084	-	<0.003
Ammonia - Salinity Adjusted	mg/L as N		13	
Unionized Ammonia - Salinity Adjusted	mg/L as N	-	0.085	-
Ammonia - at 48 Hours	mg/L as N	ND	ND	ND
Unionized Ammonia - at 48 Hours	mg/L as N	ND	ND	ND

Comments: ND= No data. 48 hour ammonia data is not available due to a technician error.

Summary of StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results. American Samoa Joint Cannery Outfall Effluent TABLE 5. Evaluation. March 2006.

Date	Species	g	6-Hour Endpo	ints
		LC-50	NOEC	LOEC
02/93 ¹	Penaeus vannami	4.8%	3.1%	6.25%
10/93 ¹	Penaeus vannami	15.67%	3.1%	6.25%
02/94 ¹	Penaeus vannami	15.76%	<1.6%	1.6%
10/94 ¹	Americamysis bahia	31.2%	25.0%	50.0%
03/95 ¹	Penaeus vannami	14.8%	6.25%	12.5%
03/95 ¹	Americamysis bahia	10.8%	6.25%	12.5%
02/96 ¹	Penaeus vannami	>50.0%	>50.0%	>50.0%
03/96 ¹	Penaeus vannami	44.4%	25.0%	50.0%
11/96 ¹	Penaeus vannami	7.11%	3.1%	6.25%
03/97 ¹	Penaeus vanņami	39.36%	12.5%	25.0%
.09/97 ¹	Penaeus vannami	12.3%	6.25%	12.5%
06/98 ¹	Americamysis bahia	17.2%	6.25%	12.5%
11/98 ¹	Americamysis bahia	15.0%	6.25%	12.5%
02/00 ¹	Americamysis bahia	20.0%	6.25%	12.5%
.08/00 ¹	Americamysis bahia	17.1%	3.1%	6.25%
03/01 ²	Americamysis bahia	13.81%	12.5%	25.0%
03/02 ²	Americamysis bahia	16.13%	12.5%	25.0%
08/02 ²	Americamysis bahia	10.23%	6.25%	12.5%
03/03 ²	Americamysis bahia	28.4%	25.0%	50.0%
08/03 ²	Americamysis bahia	43.2%	25.0%	50.0%
03/04 ²	Americamysis bahia	>50.0%	50.0%	>50.0%
10/04 ²	Americamysis bahia	>50.0%	50.0%	>50.0%
03/05 ²	Americamysis bahia	48.5%	25%	50%
10/05²	Americamysis bahia	>50.0%	50%	>50
03/06 ²	Americamysis bahia	35.6%	25%	50%

Notes:

Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California
 Assays conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A

DATA SHEETS

STATISTICAL SUPPORT

Contents	Number of Pages
Methods Used in NPDES Permit Biomonitoring Testing	1
A. bahia Acute Bioassay Data Summary	3
A. bahia Survival Statistics: LC-50, NOEC	14
A. bahia Organism Culture Sheet	1
Preparation of Dilutions	1
Record of Meters Used for Water Quality Measurements	1
Unionized Ammonia Calculation	4
Sample Receipt Record	1
Chain of Custody	1

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
Ceriodaphnia dubia, Daphnia pulex	EPA-821-R-02-012
Pimephales promelas	EPA-821-R-02-012
Americamysis bahia	EPA-821-R-02-012
Menidia beryllina, Cyprinodon variegatus	EPA-821-R-02-012
Chronic Exposure Bioassays	
Ceriodaphnia dubia	EPA-821-R-02-013, 1002.0
Pimephales promelas	EPA-821-R-02-013, 1000.0
Cyprinodon variegatus	EPA-821-R-02-014, 1004.0
Menidia beryllina	EPA-821-R-02-014, 1006.0
Arbacia punctulata	EPA-821-R-02-014, 1008.0
Champia parvula	EPA-821-R-02-014, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	EPA 310.2
Chlorine, Residual	Standard Methods 20th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20th Edition - Method 5310C
Specific Conductance	Standard Methods 20th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20th Edition - Method 4500NH3G
рН	Standard Methods 20th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20th Edition - Method 4500-O G

ACUTE BIOASSAY DATA SUMMARY

STUDY	NT: CH2M Hill TEST ORGANISM: A. bahia										,			"∆	S RE	CEIVE	ED" E	FFLL	JENT	AND	DILU	ENT (CHEM	IISTF	IES					
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 ^{★ -} See:" EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA" sheet.
 ★ - AERATE FROM START!
 ◇ - "Old" water qualities (prior to renewal)

^{☆ - &}quot;New"water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

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 ^{★ -} See:" EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA" sheet.
 + - AERATE FROM START!
 ◇ - "Old" water qualities (prior to renewal)

ACUTE BIOASSAY DATA SUMMARY

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STUDY	NT: CH2M Hill TEST ORGANISM: A. bahia															"Δ	SRE	CEIV	ED" E	FFLL	JENT	AND	DILU	ENT (CHEN	ИISTF	RIES	· · · · · ·		
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 ^{★ -} See:" EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA" sheet.
 ★ - AERATE FROM START!
 ♦ - "Old" water qualities (prior to renewal)
 ☆ - "New"water qualities (post renewal)

CETIS Test Summary

Report Date:

16 Mar-06 5:33 PM

Link: 11-3381-8273

Americamysi	s 96-h Acute Survival Tes	st					EnviroSystems, Inc.
Test No: Start Date: Ending Date: Setup Date:	07-1088-3899 08 Mar-06 03:20 PM 12 Mar-06 01:20 PM 08 Mar-06 03:20 PM	Test Type: Protocol: Dil Water: Brine:		R-02-012 (26 awater	002)	Duration: Species: Source:	94h Americamysis bahia ARO - Aquatic Research Organisms, N
1	02-2666-6345 01 Mar-06 12:00 PM : 08 Mar-06 12:00 PM 7d 3h	Material: Code: Source: Station:	Industrial E 14326 CH2M Hill- Joint Cann	American :	Samoa	Client: Project:	CH2M Hill WET Quarterly Compliance Test (1Q)
Comparison S	Summary						
Analysis	Endpoint	NOEL	LO	EL	ChV	MSDp	Method
16-8050-0151	24h Proportion Survived	100	> 100		N/A	5.33%	Steel's Many-One Rank
14-9795-7901	48h Proportion Survived	50	75		61.237	27.87%	Steel's Many-One Rank
20-2463-6746	72h Proportion Survived	25	50		35.355	6.62%	Dunnett's Multiple Comparison
05-5702-4063	96h Proportion Survived	25	50		35.355	15.68%	Steel's Many-One Rank
Point Estimate	e Summary						
Analysis	Endpoint	% Effec	t Cor	nc-%	95% LCL	95% UCL	Method
04-6135-6263	48h Proportion Survived	50	63.2	25922	58.26902	68.67678	Trimmed Spearman-Karber
13-7987-5949	72h Proportion Survived	50	35.7	5597	34.97713	36.55215	Trimmed Spearman-Karber
16-2376-3199	96h Proportion Survived	50	35.5	9903	34.61591	36.61006	Trimmed Spearman-Karber
Test Acceptab	illity						
Analysis	Endpoint	Attribu	te	Statistic	Acceptable	Range	Decision
05-5702-4063	96h Proportion Survived	Control	Response	0.92	0.9 - N/A		Passes acceptability criteria
16-2376-3199	96h Proportion Survived	Control	Response	0.92	0.9 - N/A		Passes acceptability criteria

CETIS™ v1.026C

Approval:____

Analyst:____

Report Date:

Link:

16 Mar-06 5:33 PM

CETIS Test Summary

11-3381-8273 24h Proportion Survived Summary Control Type Reps Mean Minimum Maximum SE SD C۷ 0 Lab Water 1.00000 5 1.00000 1.00000 0.00000 0.00000 0.00% 6.25 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 12.5 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 25 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 50 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 75 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 100 0.92000 0.80000 1.00000 0.03742 0.08367 9.09% 48h Proportion Survived Summary Conc-% Control Type Mean Minimum Maximum SE SD CV 0 Lab Water 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 6.25 5 0.98000 0.90000 1.00000 0.02000 0.04472 4.56% 12.5 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 25 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 50 0.78000 5 0.00000 1.00000 0.19596 0.43818 56.18% 75 5 0.44000 0.00000 0.70000 0.13266 0.29665 67.42% 100 5 0.00000 0.00000 0.00000 0.00000 0.00000 0.00% 72h Proportion Survived Summary Conc-% Control Type Reps Mean Minimum Maximum SE SD CV 0 Lab Water 5 0.94000 0.90000 1.00000 0.02449 0.05477 5.83% 6.25 5 0.98000 0.90000 1.00000 0.02000 0.04472 4.56% 12.5 5 1.00000 1.00000 1.00000 0.00000 0.00000 0.00% 25 5 0.98000 0.90000 1.00000 0.02000 0.04472 4.56% 50 5 0.02000 0.00000 0.10000 0.02000 0.04472 223.61 75 5 0.00000 0.00000 0.00000 0.00000 0.00000 0.00% 100 0.00000 0.00000 0.00000 0.00000 0.00000 0.00% 96h Proportion Survived Summary Conc-% Control Type Reps Mean Minimum Maximum SE SD CV Lab Water 5 0.92000 0.80000 1.00000 0.03742 0.08367 9.09% 6.25 5 0.92000 0.80000 1.00000 0.04899 0.10954 11.91% 12.5 5 0.94000 0.80000 1.00000 0.04000 0.08944 9.52% 25 5 0.92000 0.70000 1.00000 0.05831 0.13038 14.17% 50 5 0.02000 0.00000 0.10000 0.02000 0.04472 223.61 75 5 0.00000 0.00000 0.00000 0.00000 0.00000 0.00%

000-148-126-2

100

5

0.00000

0.00000

0.00000

0.00000

0.00000

0.00%

CETIS™ v1.026C

Analyst:__ Approval: **CETIS Test Summary**

Report Date: Link: 16 Mar-06 5:33 PM 11-3381-8273

		-					
24h Propoi	rtion Survived De	tail			1		
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water	1.00000	1.00000	1.00000	1.00000	1.00000	
6.25		1.00000	1.00000	1.00000	1.00000	1.00000	
12.5		1.00000	1.00000	1.00000	1.00000	1.00000	
25		1.00000	1.00000	1.00000	1.00000	1.00000	
50		1.00000	1.00000	1.00000	1.00000	1,00000	
75		1.00000	1.00000	1.00000	1.00000	1.00000	
100		1.00000	0.90000	1.00000	0.80000	0.90000	
48h Propor	tion Survived Det	tail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water	1.00000	1.00000	1.00000	1.00000	1.00000	 •
6.25		0.90000	1.00000	1.00000	1.00000	1.00000	
12.5		1.00000	1.00000	1.00000	1.00000	1.00000	
25		1.00000	1.00000	1.00000	1.00000	1.00000	
50		1.00000	1.00000	1.00000	0.00000	0.90000	
75		0.30000	0.50000	0.00000	0.70000	0.70000	
100		0.00000	0.00000	0.00000	0.00000	0.00000	
72h Propor	tion Survived Det	ail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water	0.90000	1.00000	0.90000	1.00000	0.90000	_
6.25		0.90000	1.00000	1.00000	1.00000	1.00000	
12.5		1.00000	1.00000	1.00000	1.00000	1.00000	
25		1.00000	1.00000	1.00000	0.90000	1.00000	
50		0.10000	0.00000	0.00000	0.00000	0.00000	
75		0.00000	0.00000	0.00000	0.00000	0.00000	
100		0.00000	0.00000	0.00000	0.00000	0.00000	
96h Proport	tion Survived Det	ail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Water	0.80000	1.00000	0.90000	1.00000	0.90000	
6.25		0.80000	1.00000	0.80000	1.00000	1.00000	
12.5		1.00000	0.90000	1.00000	0.80000	1.00000	
25		0.90000	1.00000	1.00000	0.70000	1.00000	
50		0.10000	0.00000	0.00000	0.00000	0.00000	
75		0.00000	0.00000	0.00000	0.00000	0.00000	
100		0.00000	0.00000	0.00000	0.00000	0.00000	

Comparisons: Report Date: Page 1 of 8

Analysis:

16 Mar-06 5:33 PM 05-5702-4063

Americamysis 96-h Acute Survival Test EnviroSystems, Inc. Test No: 07-1088-3899 Test Type: Survival (96h) Duration: 94h Start Date: 08 Mar-06 03:20 PM Protocol: EPA/821/R-02-012 (2002) Species: Americamysis bahia Ending Date: 12 Mar-06 01:20 PM Dil Water: Natural Seawater ARO - Aquatic Research Organisms, N Source: Setup Date: 08 Mar-06 03:20 PM Brine: Not Applicable Endpoint Analysis Type Sample Link Control Link Date Analyzed Version 96h Proportion Survived Comparison 11-3381-8273 11-3381-8273 16 Mar-06 5:22 PM CETISv1.026 Method Alt H Data Transform Z NOEL LOEL Toxic Units ChV MSDp Steel's Many-One Rank C > TAngular (Corrected) 25 50 4.00 35.355 15.68% Test Acceptability Attribute Statistic Acceptable Range Decision Control Response 0.92 0.9 - N/A Passes acceptability criteria **ANOVA Assumptions** Attribute Test Statistic Critical P Level Decision(0.01) Variances Bartlett 3.04008 13.27671 0.55114 Equal Variances Distribution Shapiro-Wilk W 0.84838 0.88746 0.00137 Non-normal Distribution ANOVA Table Source Sum of Squares Mean Square DF F Statistic P Level Decision(0.05) Between 4.896395 1.224099 4 59.64 0.00000 Significant Effect Error 0.4104991 0.020525 20 Total 5.30689383 1.2446236 24 **Group Comparisons** Control Conc-% Statistic Critical P Level Ties Decision(0.05) Lab Water 6.25 28 17 > 0.0500 3 Non-Significant Effect 12.5 29.5 17 > 0.0500 3 Non-Significant Effect 25 29 17 > 0.0500 2 Non-Significant Effect 50 15 17 <= 0.0500 3 Significant Effect **Data Summary Original Data** Transformed Data Conc-% Control Type Mean Count Minimum Maximum SD Mean Minimum Maximum SD 0 Lab Water 5 0.92000 0.80000 1.00000 0.08367 1.28585 1.10715 1.41202 0.12892 6.25 5 0.92000 0.80000 1.00000 0.10954 1.29007 1.10715 1.41202 0.16698 12.5 5 0.94000 0.80000 1.00000 0.08944 1.31845 1.10715 1.41202 0.13759 25 5 0.92000 0.70000 1.00000 0.13038 1.29525 0.99116 1.41202 0.18406 50 5 0.02000 0.00000 0.10000 0.04472 0.19137 0.15878 0.32175 0.07288 Data Detail Conc-% **Control Type** Rep 1 Rep 2 Rep 3 Rep 4 Rep 5 Rep 6 Rep 7 Rep 8 Rep 9 Rep 10 Lab Water 0.80000 1.00000 0.90000 1.00000 0.90000 6.25 0.80000 1.00000 0.80000 1.00000 1.00000 12.5 1.00000 0.90000 1.00000 0.80000 1.00000 25 0.90000 1.00000 1.00000 0.70000 1.00000 50 0.10000 0.00000 0.00000 0.00000 0.00000

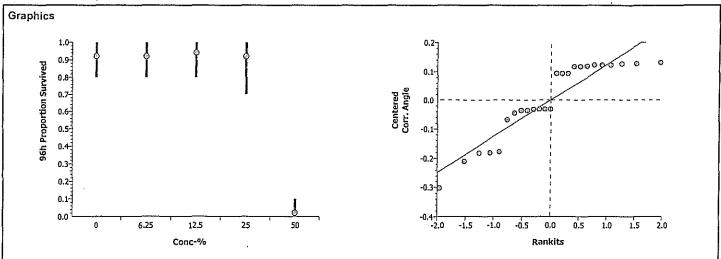
000-148-126-2

CETIS™ v1.026C

Analyst:_____ Approval:____

Comparisons: Report Date: Page 2 of 8 16 Mar-06 5:33 PM

Analysis: 05-5702-4063



Comparisons: Report Date: Page 3 of 8 16 Mar-06 5:33 PM

Analysis:

14-9795-7901

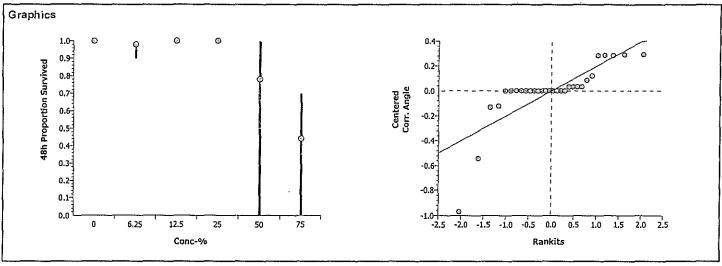
Americamysi	s 96-h Acute Su	ırvival Te	st						Enviro	Systems, Inc.
Test No: Start Date: Ending Date: Setup Date:	07-1088-3899 08 Mar-06 03: 12 Mar-06 01: 08 Mar-06 03:	20 PM	Test Type: Protocol: Dil Water: Brine:	Survival (96 EPA/821/R- Natural Sea Not Applica	-02-012 (200) awater	2)	Duration Species: Source:	Americamy		Organisms, N
Endpoint		Ana	ilysis Type		Sample I			te Analyzed	Version	1 .
48h Proportion	Survived	Con	nparison		11-3381-	8273 11-33	381-8273 16	Mar-06 5:22 F	PM CETISV	1.026
Method		Alt	H Data T	ransform	Z	NOEL	LOEL To	oxic Units	ChV	MSDp
Steel's Many-C	One Rank	C >	T Angula	r (Corrected)	50	75 2.	00	61.237	27.87%
ANOVA Assu	mptions									
Attribute	Test			Statistic	Critical	P Leve	l Deci	ision(0.01)		
Variances	Modified	Levene		2.41905	3.89507	0.06536	6 Equa	al Variances		
Distribution	Shapiro-	Wilk W		0.67006	0.89981	0.00000	0 Non-	normal Distrib	ution	
ANOVA Table										
Source	Sum of	Squares	Mean Squa	re DF	F Statist	ic PLeve	l Deci	sion(0.05)		
Between	2.054252	2	0.4108503	5	5.80	0.00120		ficant Effect		
Error	1.701514	1	0.0708964	24						
Total	3,755765	58	0.4817467	29						
Group Compa	risons									
Control	vs Conc-%		Statistic	Critical	P Level	Ties	Deci	sion(0.05)		
Lab Water	6.25		25	16	> 0.0500	1	Non-	Significant Eff	ect	
	12.5		27.5	16	> 0.0500	1	Non-	Significant Eff	ect	
	25		27.5	16	> 0.0500	1		Significant Eff		
	50		22.5	16	> 0.0500	1		Significant Eff	ect	:
	75		15	16	<= 0.0500	2	Signi	ficant Effect		
Data Summar	у	_		Origi	inal Data			Transfo	rmed Data	
	Control Type	Count	Mean	Minimum	Maximum		Mean	Minimum	Maximum	SD
	Lab Water	5	1.00000	1.00000	1.00000	0.00000	1.41202	1.41202	1.41202	0.00026
6.25	•	5	0.98000	0.90000	1.00000	0.04472	1.37942	1.24905	1.41202	0.07288
12.5		5	1.00000 .	1.00000	1.00000	0.00000	1.41202	1.41202	1.41202	0.00026
25		5	1.00000	1.00000	1.00000	0.00000	1.41202	1.41202	1.41202	0.00026
50 75		5 5	0.78000 0.44000	0.00000	1.00000 0.70000	0.43818 0.29665	1.12877 0.70123	0.15878 0.15878	1.41202 0.99116	0.54682 0.34794
								0.,007.0		0.047.04
Data Detail Conc-%	Control Type	Par 1	Par 2	Dan ?	Pan 4 I	Dan S D.	an 6 De-	7 Dan 0	Don 0	Pan 40
	Control Type Lab Water	Rep 1 1.00000	Rep 2 1.00000	Rep 3 1.00000		Rep 5 Re	ep 6 Rep	7 Rep 8	Rep 9	Rep 10
6.25	225 (1010)	0.90000	1.00000			.00000				
12.5		1.00000	1.00000			.00000				
25		1.00000	1.00000			.00000				
			1.00000			0.90000				
50		1.00000	1.00000	1.00000	0.00000	1,30000				

Analyst:____

Comparisons: Report Date: Page 4 of 8 16 Mar-06 5:33 PM

Analysis:

14-9795-7901



Analyst:____

Comparisons: Report Date: Page 5 of 8 16 Mar-06 5:33 PM

Analysis:

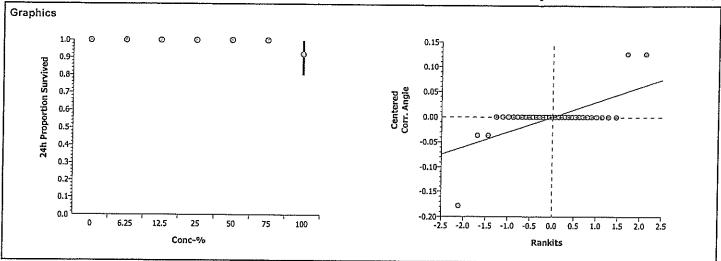
16-8050-0151

Americamys	is 96-h Acute S	rvival Te	et						Enviro	Systems, Inc.
									FIIAITO	
Test No: Start Date: Ending Date: Setup Date:	07-1088-3899 08 Mar-06 03: 12 Mar-06 01: 08 Mar-06 03:	20 PM 20 PM	Test Type: Protocol: Dil Water: Brine:		-02-012 (200 awater	2)	Durat Speci Sourc	es: Americam	iysis bahia uatic Researci	n Organisms,
Endpoint		Ana	lysis Type		Sample		trol Link	Date Analyzed	Versio	
24h Proportio	n Survived	Con	parison		11-3381-	-8273 11-3	381-8273	16 Mar-06 5:22	PM CETIS	/1.026
Method		Alt	H Data T	ransform	Z	NOEL	LOEL	Toxic Units	ChV	MSDp
Steel's Many-	One Rank	C >	T Angula	ar (Corrected)	100	>100	1.00	N/A	5.33%
ANOVA Assu	ımptions									
Attribute	Test			Statistic	Critical	P Leve	ei D	ecision(0.01)		•
Variances	Modified	Levene		12.20194	3.52756	0.0000	00 U	nequal Variance	s	
Distribution	Shapiro-	Wilk W		0.48733	0.91004	0.0000)0 N	on-normal Distri	bution	
ANOVA Table	е								· · · · · · · · · · · · · · · · · · ·	, , , , , , , , , , , , , , , , , , ,
Source	Sum of	Squares	Mean Squa	re DF	F Statis	tic P Leve	el D	ecision(0.05)		
Between	0.06821	47	0.0113691	6	4.79	0.0017	'8 S	ignificant Effect		
Error	0.06647	90	0.0023743	28						
Total	0.13469	368	0.0137434	34		<u> </u>				
Group Comp	arisons									
Control	vs Conc-%		Statistic	Critical	P Level	Ties	D	ecision(0.05)		
Lab Water	6.25		27.5	16	> 0.0500	1	N	on-Significant Ef	fect	
	12.5		27.5	16	> 0.0500	1	Ν	on-Significant Ef	fect	
	25		27.5	16	> 0.0500	1	N	on-Significant Ef	fect	
	50		27.5	16	> 0.0500	1	N	on-Significant Ef	fect	
	75		27.5	16	> 0.0500	1	N	on-Significant Ef	fect	
	100		20	16	> 0.0500	2	N	on-Significant Ef	fect	
Data Summa	гу	_		Origi	nal Data			Transfo	ormed Data	
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Lab Water	5	1.00000	1.00000	1.00000	0.00000	1.41202		1.41202	0.00026
6.25		5	1.00000	1.00000	1.00000	0.00000	1.41202		1.41202	0.00026
12.5		5	1.00000	1.00000	1.00000	0.00000	1.41202		1.41202	0.00026
25		5	1.00000	1.00000	1.00000	0.00000	1.41202		1.41202	0.00026
50		5	1.00000	1.00000	1.00000	0.00000	1.41202		1.41202	0.00026
75 100		5 5	1.00000 0.92000	1.00000 0.80000	1.00000 1.00000	0.00000 0.08367	1.41202 1.28585		1.41202 1.41202	0.00026 0.12892
				3.0000		0.00001	1,20000	1,10/10	1.71202	U. 14004
Data Detail										
Conc-%	Control Type	Rep 1	Rep 2				ep6 R	ep7 Rep8	Rep 9	Rep 10
0	Lab Water	1.00000	1.00000			1.00000				
6.25		1.00000	1.00000			1.00000				
12.5		1.00000	1.00000			1.00000				
25 50		1.00000	1.00000			1.00000				
50 75		1.00000	1.00000			1.00000				
75 100		1.00000	1.00000			00000				
100		1.00000	0.90000	1.00000	0.80000	0.90000		. ,		

Analyst:___

Comparisons: Report Date: Page 6 of 8 16 Mar-06 5:33 PM





Analyst:_

CETIS Analysis Detail

Comparisons: Report Date: Page 7 of 8 16 Mar-06 5:33 PM

Analysis:

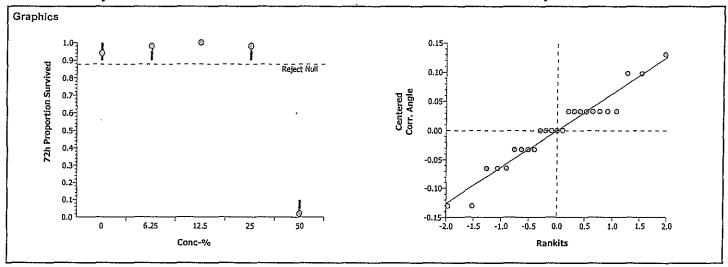
20-2463-6746

Americamys	is 96-h Acute S	urvival Te	st								Enviro	Systems, Inc.
Test No: Start Date: Ending Date Setup Date:	07-1088-3899 08 Mar-06 03: 12 Mar-06 01: 08 Mar-06 03:	20 PM 20 PM	Test Type: Protocol: Dil Water: Brine:	Survival (96 EPA/821/R Natural Sea Not Applica	-02-012 (200 awater	02)		Dura Spec Sour		94h Americamy ARO - Aqu		Organisms, N
Endpoint		Ana	lysis Type		Sample	Link	Contr	ol Link	Date	Analyzed	Versio	1
72h Proportio	n Survived	Com	parison		11-3381	-8273	11-33	81-8273	16 M	ar-06 5:22 F	PM CETIS	1.026
Method		Alt	H Data 1	ransform	Z	NO	EL	LOEL	Tox	ic Units	ChV	MSDp
Dunnett's Mu	tiple Comparisor	C >	T Angula	ar (Corrected)	25		50	4.00		35.355	6.62%
ANOVA Assi	ımptions				<u> </u>							
Attribute	Test			Statistic	Critical		P Level		Decisi	on(0.01)		
Variances	Modified	Levene		1.15385	4.43069		0.36047	7	Equal \	/ariances		
Distribution	Shapiro-	Wilk W		0.94255	0.88746	3	0.17907	7	Normal	Distribution	1	
ANOVA Tabl	9						· - /					
Source	Sum of	Squares	Mean Squa	re DF	F Statis	stic	P Level		Decisio	on(0.05)		
Between	5.59388		1.39847	4	292.53	···-	0.00000)	Signific	ant Effect		
Error	0.09561	36	0.0047807	20	_							
Total	5.68949	377	1.4032507	24								
Group Comp	arisons											
Control	vs Conc-%		Statistic	Critical	P Level		MSD	i	Decisio	on(0.05)		
Lab Water	6.25		-1.4907	2.3	> 0.0500		0.10058	}	Non-Siç	gnificant Eff	ect	
	12.5		-2.2361	2.3	> 0.0500	ļ	0.10058		Non-Sig	gnificant Eff	ect	(
	25		-1.4907	2.3	> 0.0500		0.10058		Non-Sig	gnificant Eff	ect	
	50		25.6774	2.3	<= 0.0500		0.10058		Signific	ant Effect		
Data Summa	ry			Orig	inal Data					Transfo	rmed Data	
Conc-%	Control Type	Count	Mean	Minimum	Maximum			Mean		Minimum	Maximum	SD
0	Lab Water	5	0.94000	0.90000	1.00000	0.0	5477	1.3142	23	1.24905	1.41202	0.08926
6.25		5	0.98000	0.90000	1.00000	0.0	4472	1.3794	12	1.24905	1.41202	0.07288
12.5		5	1.00000	1.00000	1.00000	0.0	0000	1.4120)2	1.41202	1.41202	0.00026
25		5	0.98000	0.90000	1.00000	0.0	4472	1.3794	12	1.24905	1.41202	0.07288
50		5	0.02000	0.00000	0.10000	0.0	4472	0.1913	37	0.15878	0.32175	0.07288
Data Detail							,					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3		Rep 5		p 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	0.90000	1.00000	0.90000		0.9000						
6.25		0.90000	1.00000	1.00000		1.0000						
12.5		1.00000	1.00000	1.00000		1.0000						1
25		1.00000	1.00000	1.00000		1.0000						ļ
50		0.10000	0.00000	0.00000	0.00000	0.0000	0					

Analyst:_____

CETIS Analysis Detail

Comparisons: Report Date: Analysis: Page 8 of 8 16 Mar-06 5:33 PM 20-2463-6746



Spearman-Karber:

Report Date:

Page 1 of 3

Analysis:

16 Mar-06 5:33 PM 04-6135-6263

EnviroSystems, Inc.

CETIS Analysis Detail

Ending Date: 12 Mar-06 01:20 PM

Test No:

Start Date:

Americamysis 96-h Acute Survival Test

07-1088-3899

08 Mar-06 03:20 PM

Test Type: Survival (96h)

Protocol: EPA/821/R-02-012 (2002)

Dil Water: Natural Seawater

Duration: 94h

Species: Americamysis bahia

Source:

ARO - Aquatic Research Organisms, N

Setup Date: 08 Mar-06 03:20 PM Not Applicable Analysis Type Sample Link Endpoint

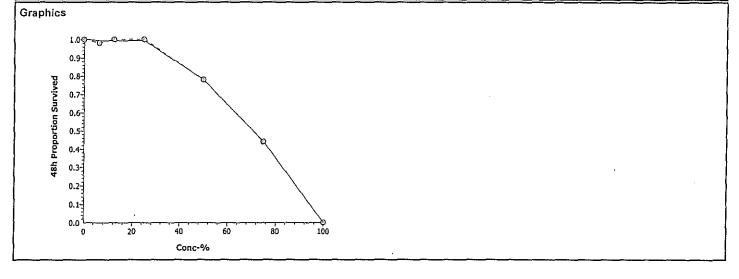
Brine:

Control Link Date Analyzed Version 11-3381-8273 11-3381-8273 Trimmed Spearman-Karber 16 Mar-06 5:30 PM 48h Proportion Survived CETISv1.026

Spearman-Karber Options Point Estimates

Threshold Option Lower Threshold Trim Level Sigma EC50/LC50 95% LCL 95% UCL Control Threshold 0 0.67% 1.801124 0.01784305 63.25922 58.26902 68.67678

Data Summary			Calculated Variate(A/B)							
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SE	SD	A	В	
0	Lab Water	5	1.00000	1.00000	1.00000	0.00000	0.00000	50	50	
6.25		5	0.98000	0.90000	1.00000	0.00913	0.04472	49	50	
12.5		5	1.00000	1,00000	1.00000	0.00000	0.00000	50	50	
25		5	1.00000	1.00000	1.00000	0.00000	0.00000	50	50	
50		5	0.78000	0,00000	1.00000	0.08944	0.43818	39	50	
75		5	0.44000	0.00000	0.70000	0.06055	0.29665	22	50	
100		5	0.00000	0.00000	0.00000	0.00000	0.00000	0	50	



Spearman-Karber:

Page 2 of 3

Report Date:

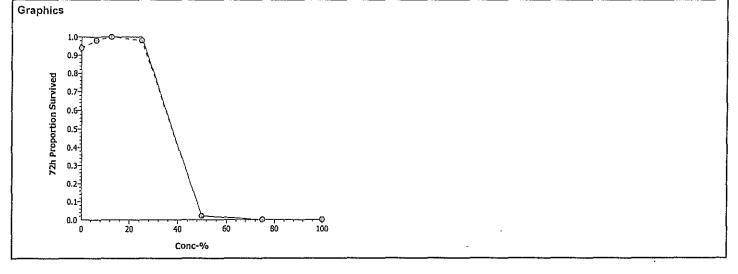
16 Mar-06 5:33 PM

Analysis:

13-7987-5949

CETIS Analysis Detail Americamysis 96-h Acute Survival Test EnviroSystems, Inc. Test No: 07-1088-3899 Test Type: Survival (96h) Duration: 94h Protocol: EPA/821/R-02-012 (2002) Start Date: 08 Mar-06 03:20 PM Species: Americamysis bahia Ending Date: 12 Mar-06 01:20 PM Dil Water: Natural Seawater Source: ARO - Aquatic Research Organisms, N Setup Date: Brine: Not Applicable 08 Mar-06 03:20 PM Endpoint Analysis Type Sample Link Control Link Date Analyzed Version 16 Mar-06 5:29 PM 11-3381-8273 11-3381-8273 CETISv1.026 72h Proportion Survived Trimmed Spearman-Karber Spearman-Karber Options Point Estimates 95% LCL Threshold Option Lower Threshold Trim Level Sigma EC50/LC50 95% UCL Mυ 35.75597 34.97713 Control Threshold 0.06 0.00% 1.553349 0.004782179 36.55215

Data Summary			Calculated Variate(A/B)				_			
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SE	SD	Α	В	
0	Lab Water	5	0.94000	0.90000	1.00000	0.01118	0.05477	47	50	
6.25		5	0.98000	0.90000	1.00000	0.00913	0.04472	49	50	
12.5		5	1.00000	1.00000	1.00000	0.00000	0.00000	50	50	
25		5	0.98000	0.90000	1.00000	0.00913	0.04472	49	50	
50		5	0.02000	0.00000	0.10000	0.00913	0.04472	1	50	
75		5	0.00000	0.00000	0.00000	0.00000	0.00000	0	50	
100		5	0.00000	0.00000	0.00000	0.00000	0.00000	0	50	



Spearman-Karber:

Page 3 of 3

CETIS	Analysis D	etail							oort Date: ilysis:	16 Mar-06 5:33 PN 16-2376-3199
American	nysis 96-h Acute	Survival 1	est	·						EnviroSystems, Inc.
Test No: Start Date Ending Date Setup Date	ate: 12 Mar-06 0	3:20 PM 1:20 PM	Test Ty Protoco Dil Wate Brine:		R-02-012 (200 eawater	2)	Dura Spec Sour	cies: /	94h Americamysis ARO - Aquatic	bahia Research Organisms, N
Endpoint			nalysis Typ		Sample		ntrol Link	Date A	Analyzed	Version .
96h Propo	rtion Survived	Tr	immed Spea	rman-Karber	11-3381-	8273 11	-3381-8273	16 Ma	r-06 5:27 PM	CETISv1.026
Spearman Threshold Control Th		r Thresho	old Trim 0.00%		Sig	ma)6081175	Point Estin EC50/LC50 35.59903		95% LCL 34.61591	95% UCL 36.61006
Test Acce		St	atistic	Acceptable R						00.07.000
Control Re	sponse	0.9	92	0.9 - N/A	Pass	es accepta	bility criteria			
Data Sum	mary			Calcu	lated Variate	(A/B)				
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SE	SD	A	В	
0	Lab Water	5	0.92000	0.80000	1.00000	0.01708	0.08367	46	50	
6.25		5	0.92000	0.80000	1.00000	0.02236	0.10954	46	50	ļ
12.5		5	0.94000	0.80000	1.00000	0.01826	0.08944	47	50	
25		5	0.92000	0.70000	1.00000	0.02661	0.13038	46	50	
50		5	0.02000	0.00000	0.10000	0.00913	0.04472	1	50	
75		5	0.00000	0.00000	0.00000	0.00000	0.00000	0	50	
100		5	0.00000	0.00000	0.00000	0.00000	0.00000	0	50	
Graphics										
96h Proportion Survived	1.0 0.9 0.8 0.7- 0.6- 0.5- 0.4									

Analyst:___

Conc-%



Aquatic Research Organisms

DATA SHEET

I.	Organism I	History
	Species:	AMERICAMYSIS bahin
٠	Source:	Lab reared Hatchery reared Field collected
		Hatch date 3-3-06 Receipt date
		Lot number 030306 MS Strain
		Brood Origination Flouish
II.	Water Qua	lity
		Temperature 25°C Salinity ~30 ppt DO
		pH_7.8 Hardness ppm
III.	Culture Co	
		System: ZEC/RC
		Diet: Flake Food Phytoplankton Trout Chow
	•	Brine Shrimp Rotifers Other Europe Shrings ET
		Prophylactic Treatments:
		Comments:
IV	. Shipping I	Information
		Client: ESI # of Organisms: 350+
		Carrier: Date Shipped: 3-7-06
Bi	ologist:	Mark Dosengard

1 - 800 - 927 - 1650

EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA

PARAMETER	100% Effluent	50% Effluent	Diluent - Lab Salt
TRC	<0.05		۷0,05
As Received - pH (SU) @ 20°C	6.46		7.87
As Received - Salinity (ppt)	11.4		25
As Received - Dissolved Oxygen (mg/L)‡	1,3		7.3
As Received - Ammonia (pull)	-002		- 605
Salinity Adjusted - pH (SU) @ 20°C	7.07	(3) 2.27 7.22	
Salinity Adjusted - Salinity (ppt)	24	25	
After Aeration - Dissolved Oxygen (mg/L)	3.6	@ 5-8 518	
Salinity Adjusted - Ammonia (pull)		-003	And the second s
48 hour Ammonia (pull)			
48 hour pH (SU) @ 20°C		7.27	7.95.

⁻ Aerate prior to mixing concentrations.

PREPARATION OF DILUTIONS

THE ARATION OF DIEGHORS									
STUDY: 1432	26	CLIENT: CH2M HILL - American Samoa							
SPECIES: A. bah	ia		1						
Diluent:	Day: O		Day: 2				-		
Lab Salt	Sample: EC	A	Sample:				-		
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol	HRS	Date	Time	Initials	
LAB	D	1000	0	1000800	0	3/8/06	1430	SJ	
6.25%	62.5		90		48	3-10-06	1430	AF	
12.5%	125		[OD		Comm	ents:	· .		
25%	250		200					`	
50%	500		400						
75%	750		600						
100% ′	1000	X	800	A					

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: \437	2C	CLIENT: CH2M HILL - American Samoa							
		WATER	QUALITIES - A	bahia					
HOURS:	0	24	48 - old	48 - new	72	96			
Water Quality Station #		2	1	Ì					
Initials	SJ	WO	AF	Af					
Date	3/8/04	3/9/06	3-10-66	7					

Water Quality	Station #1	Water Quality	Station #2	COMMENTS	
DO meter#	18	DO meter#	19		
DO probe #	12	DO probe #	1/		
pH meter#	1097	pH meter#	470		
pH probe #	44	pH probe #	45		
S/C meter#	45F 30B	S/C meter#	451 30B		
S/C probe #		S/C probe #	1		
Salinity meter#	1	Salinity meter#	\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \		

STUDY: 14309

CLIENT: CH2MHill - American Samoa PROJECT: Wastewater Treatment Plant TASK: Unionized Ammonia Calculations

		Sample					
Day / Date	Treatment	Temperature Deg C	pH SU	NH₃ mg/L	NH₃ mg/L		
Day 0	Lab Diluent 50% Effluent	20 20	7.87 7.22	0.1 13.0	0.003 0.085		
	100% Effluent	20	6.46	23.4	0.027		

Report No:

14309

SDG:

Project:

American Samoa

Sample ID:

14309-005 ESIN1 LAB SALT 25 ppt

ND

Matrix: Sampled: Water

03/08/06

Parameter

Result Quant Limit

Units

Date Prepared

Date of Analysis

Method/Reference

Ammonia-N

14309-005

0.1

mg/L as N

03/10/06

03/10/06

SM 4500-NH3 G

Notes:

ND = Not Detected

ESI

Report No: Project: 14326 SDG: American Samoa Sample ID: **EFFLUENT 100%** Matrix: Water Sampled: 03/08/06 Parameter Result Quant Units Date Date of Method/Reference Limit Prepared Analysis 0.1 Ammonia-N 14326-002 23.4 mg/L as N 03/27/06 03/27/06 SM 4500-NH3 G Notes:

Report No:

14326

SDG:

Project:

American Samoa **EFFLUENT 50%**

Sample ID: Matrix:

Water

Sampled:

03/08/06

Parameter

Result

Units

Quant

Limit

Date Prepared

Date of Analysis Method/Reference

Ammonia-N

14326-003

13

0.1 mg/L as N 03/10/06

03/10/06

SM,4500-NH3 G

Notes:

EnviroSystems, Inc.
One Lafayette Road
P.O. Box 778
Hampton, N.H. 03843-0778
(603) 926-3345 · (603) 926-3521 Fax
www.envirosystems.com

SAMPLE RECEIPT RECORD FOR CHRONIC TOXICITY EVALUATIONS

STUDY#: 14326		CLIENT: Ameri	Can Samoa							
	SAMPLE R	ECEIPT INFORMA	TION							
	Start Sample	First Renewal	Second Renewal	Third Renewal						
Sample Receipt Date & Time:	3/8/06 /240									
Received By:	SU									
Delivered Via:	Fed Ex UPS Client Courier ESI									
Logged Into Lab By:	SJ			. /						
Date &Time Logged In:	3/8/06 1240			126						
SAMPLE CONDITION INFORMATION										
Chain of Custody?	(res)or No	Yes or No	Yes or No	Yes or No						
Chain of Custody Signed?	(res or No	Yes or No	Yes or No	Yes or No						
Chain of Custody Complete?	(Yes) or No	Yes or No	Yes or No	Yes or No						
Sample Date?	(Yes) or No	Yes or No	Yes or No	. Yes or No						
Sample Time?	(Yes) or No	Yes or No	Yes of No	Yes or No						
Sample Type?	(Yes) or No	Yes or No	Yes or No	Yes or No .						
Custody Seal in Place?	Yes or No	Yes or No	yes or No	Yes or No						
Shipping Container Intact?	(Yes) or No	Yes or No	Yes or No	Yes or No						
Temp Blank Temperature:	0°C									
DOES CLIENT NEED NOTIFICATION OF TEMP?	· · Yes or No	Yes or No	Yes or No	Yes or No						
Sample Arrived on Ice?	(Yes) or No	Yes or No	Yes or No	Yes or No						
COMMENTS:	1×59 EFF			,						
	<i>y</i> J									
		/								
		/								

APPLIED SCIENCES LABORATORY CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES CH2M Hill Project # LABTEST CODES * *** * * * * * * SHADED AREA FOR LAB USE ONLY Purchase Order # 147323,JC,06, KT ĹĹĹĹĽĽĽĽĽĽĽĽĽĽĽĽĽ Project Name JOINT CANNED OUTFALL Kit Request# Company Name/CH2M HILL Office 0 CHZM HILL F **ANALYSES REQUESTED** Project # 👊 🧼 🙃 Project Manager & Phone # Report Copy to: С Mr. DX SAME 0 B STEVE CUSTA Ms.[] 707-677-0123 No. of Samples Sample Disposal: Requested Completion Date: Sampling Requirements 0 3 SDWA NPDES RCRA OTHER Return M **K** Ν LIMS Ver Ε Matrix Туре R GRAB S A O I I R COMP W S Sampling A T E R CLIENT SAMPLE ID (9 CHARACTERS) 96 LAB 2 REMARKS L į iĎ., Time · ID · Date Ø6NT Date/Time Release in and print name) Date/Time gn and print name) Relinquished By 3/1/2006 QC Level: 1 2 3 Other: . Date/Time Received By Relinquished By Date/Time 3/8/06 Received By Date/Time Relinquished By Date/Time (Flease sign and print name) (Please sign and print name)

(Please sign and print name)

(Please sign and print name)

Date/Time

Shipped Via

UPS BUS Fed-Ex

Hand

Remarks # DILUTIONS @ 100, 75, 50, 25, 12.5, 6.25 % EFFLUENT

Received By

Work Authorized By

Shipping # 782 -0788 -485